REMARKS

I. CLAIM REJECTIONS - 35 U.S.C. § 112

A. Claims 1, 4-10, 19, 20-24, 33, 35, 37 and 38

Claims 1, 4-10, 19, 38, 20-24, 33, 35, and 37 were previously rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, have possession of the claimed invention. The Examiner stated "the new limitations of 'identifying an animal which possesses a genotype indicative of a phenotypic trait' in claim 1, 'identifying an animal which possesses a genotype indicative of a significantly correlated phenotypic trait' in claim 20, 'selecting animals which possess a desired MC4R genotype indicative of a significantly associated phenotypic trait' in claim 33, 'has previously been shown to be significantly correlated with a phenotypic trait' in claim 35 and 'said polymorphism being one which has been shown to be significantly statistically associated with a phenotypic trait' in claim 37 appear to represent new matter." *PTO Paper* dated June 9, 2003 at p. 2.

Applicants amended the claims by deleting this new subject matter, thereby alleviating this rejection.

B. Claims 1, 4-10, 19-24, 29-38 and 40

Claims 1, 4-10, 19-24, 29-38, and 40 were previously rejected under 112, second paragraph, as being indefinite. The Examiner stated "claim 1 was indefinite over the recitation 'has previously been shown to be significantly associated' because it is unclear when such a

showing should or would have happened. . . . Furthermore, it was unclear what was meant by 'significantly' associated . . . ." *Id.* at p. 4

Claim 1 was amended by deleting the recitation "has previously been shown to be significantly associated" to alleviate this rejection.

Applicants amended claims 35, 37, 38 and 40 by deleting the recitations of "previously shown and/or significantly associated" phenotypic traits to alleviate this rejection.

Claim 19 was previously rejected as being indefinite. The Examiner stated "the position of a nucleotide (by number) is entirely dependent on the primer pair used in an amplification reaction and claim 19 does not specifically recite a primer pair used. Amendment of the claim to recite 'wherein said assaying is carried out by an amplification step using primers SEQ ID NO: 6 and SEQ ID NO: 7, and wherein said polymorphism is at position 678 of the amplification product produced by primers SEQ ID NO: 6 and SEQ ID NO: 7' would obviate these rejections." *Id.*

In accordance with Examiner's suggestions, Applicants amended claim 19 to recite "the method of claim 9 wherein said polymorphism is at position 678 of the amplification product produced by primers SEQ ID NO:5 and SEQ ID NO:6" to alleviate this rejection. Moreover, Applicants submitted that SEQ ID NO: 6 and 7 should be recited as SEQ ID NO: 5 and 6, respectively.

Claim 20 was previously rejected as being indefinite over the recitation "a significantly correlated" and "a significantly associated" because it was unclear what it was meant to be "significantly" correlated or associated. *Id.* at pp. 4-5.

Claim 20 was amended by deleting the recitation "a significantly correlated" and "a significantly associated" from the claim to alleviate this rejection.

Further, the Examiner stated claim 33 also recited the language "a significantly associated" and claim 35 recited "a significantly correlated" and thus these claims were also indefinite for this recitation.

Applicants amended claims 33 and 35 by deleting the recitation "significantly associated" and "significantly correlated" respectively to alleviate this rejection.

Claims 22 and 23 were previously rejected under 35 U.S.C. § 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. *Id.* at p. 5.

Applicants amended claims 22 and 23 by adding a step which results in a production of the gene fragments to alleviate this rejection.

Claims 30-31 were previously rejected as being indefinite over the recitation "one which is known". *Id.*

Applicants amended claim 30 by deleting the recitation "one which is known" to alleviate this rejection.

Additionally, the Examiner stated "claims 34 and 35 recite 'polymorphism in the MC4R gene of the sample, being a G to A' and this language is confusing because it appears to state that the sample is a G to A point mutation. Amendment of the claims to read 'polymorphism in the MC4R gene of the sample, said polymorphism being a G to A' would overcome this rejection." *Id.* at pp. 5-6.

Applicants amended claims 34 and 35 according to Examiner's suggestion.

C. Claims 1, 4-10, 19-24, 29-38 and 40

Claims 1, 4-10, 19-24, 29-38, and 40 were previously rejected under 35 U.S.C. § 112, first paragraph, for enablement. The Examiner stated "the specification does not reasonably

provide enablement for methods which screen other animals, or methods which utilize other polymorphisms or methods which identify pigs that would produce meat with any other favorable meat qualities or methods which identify/screen for any animal having any possible phenotypic trait, particularly, the specification is not enabling for the detection of pigs that will produce meat with favorable marbling." *Id.* at pp. 6-7.

Applicants cancelled claim 38. Applicants amended claims 1, 4-10, 19-24, 29-37 to be drawn to a method of identifying a specific polymorphism associated with specific meat traits in animals. Because of the relatively close evolutionary link between pigs and other meat species it can be predicted that variation in this gene is also likely to be associated with meat quality in these other species. The polymorphism in the MC4R gene can be identified using the same approach set out in the application and the resulting SNPs used for association analysis. Moreover, a multiple alignment of the predicted amino acid sequences of the pig MC4R with MC4R proteins from other species, other MCR proteins or representative G-protein coupled receptors, showed that the aspartic acid found at position 298 of the seventh transmembrane domain is very highly conserved in the MCR proteins (see Figure 7).

Additionally, it was submitted that the failure to produce a significant association did not mean that a polymorphism was not indicative of favorable marbling and did not mean that the polymorphism was not useful in an analysis of the gene and trait at issue. Applicants have shown that all these significant effects of marker genotype are associated with allele 2. Allele 2 as disclosed on page 10 of the specification shows a guanine is substituted with an adenine at position 678 of the PCR product shown in Fig. 1. While the effect is not statistically significant, the trend is in that direction and the failure to reach significance may simply reflect the unequal genotype frequencies and the combination of lower mean values and high standards of errors for

this trait in this dataset. Applicants stated clearly on page 23 that the effect of loin marbling is a desirable trend. Moreover, while the size of the effects observed between genotypes is small in the commercial arena, they are significant in terms of differences in meat quality in the breeding arena. In addition, Applicants have shown that there is an association between this polymorphism and marbling. While not statistically significant, Applicants have shown an association, nonetheless.

Additionally, the Examiner stated "the prior art does not provide any evidence that this particular polymorphism is associated with all measures of meat quality." *Id.* at p. 7.

Applicants amended the claims to certain meat quality traits (i.e., pH, marbling, color, and drip loss). Moreover, Applicants have shown that a polymorphism in the MC4R gene has been located and this genetic variability is associated with phenotypic differences in meat quality traits. More specifically, Applicants have shown that the marker for improved meat characteristics as evidenced by four meat quality measurements (e.g., pH, marbling, color and drip loss) observed in the specification is associated with allele 2. As stated previously, it is believed that because of the evolutionary link between pigs and other meat-producing species, it can be predicted that variation in this gene is also likely to be associated with meat quality in these other species. This polymorphism can be identified in the MC4R gene of these species using the same approach set out in the specification and the resulting single nucleotide polymorphism used for association analysis. Applicants claim all animals having this particular polymorphism in order to have sufficient claim scope coverage because of the advent of improved methods in genetics, molecular biology, and animal breeding which would enable a rogue animal breeder to avoid the sought after claim scope coverage herein.

To the extent that genes are conserved among species and animals, it is expected that the different alleles will also correlate with variability in certain gene(s) as well as in economic or meat-producing animal species such as cattle, sheep, chicken, etc. There are instances of conserved polymorphisms among species. For example, Nonneman et al. recently discovered a polymorphism in exon 2 of the porcine TBG gene that results in the amino acid change of the consensus histidine to an asparagine. This SNP resides in the ligand-binding domain of the mature polypeptide and the Meishan allele is the conserved allele found in human, bovine, sheep and rodent TBG. Mutations in this region of human TBG result in decreased heat stability and affinity for ligand. Functional studies indicate altered binding characteristics of the TBG isoforms. Nonneman et al., *Plant & Animal Genomes XII Conference*, "Functional Validation of A Polymorphism for Testis Size on the Porcine X Chromosome", January 10-14, 2004, Town & Country Convention Center, San Diego, CA. Additionally, Winter et al. finds that increased milk fat content in different breeds is strongly associated with a lysine at position 232 of the protein encoded by bovine DGAT. An alignment of DGAT1 amino acid sequences of different plant and animal species indicates a conserved lysine residue at position 232 of the bovine sequence. Winter et al., *Proc Natl Acad Sci U S A.* July 9; 99 (14: 9300–9305 (2002). Furthermore, a conserved mutation in the MATP gene has been identified, which causes the cream coat colour in the horse. This conserved mutation was also described in mice and humans, but not in medaka. Mariat et al., *Genet Sel Evol.* Jan-Feb;35(1):119-33 (2003).

There have also been instances of conservation of a gene across species. Many genes involved in fundamental biological processes have been conserved as species have evolved, i.e., many genes are similar in different species. The MC1-R gene has been indicated to be a well-conserved gene having no other fundamental function beside pigmentation. In several species,

mutations in the MC1-R gene have been shown to cause the dominant expression of black pigment. Klungland et al., *Pigmentary Switches in Domestic Animal Species* Annals of the New York Academy of Sciences, 994:331-338 (2003). A specific protein-DNA interaction was found to be blocked by a single base pair change in the binding site of glucocorticoid receptor protein (GCR). Moreover it is reported that all three putative domains (the steroid binding, immunoreactive, and DNA binding) have been conserved between two divergent species, pig and rat. Marks et al., *J Steroid Biochem.* Jun;24(6):1097-103 (1986).

An example of a conserved gene order is demonstrated by Seroude et al. (*Mammalian Genomics*, Jun; 10(6) 565-8 (1999)) wherein a radiation hybrid map of the Chromosome 15q2.3-q2.6 region containing the RN gene was constructed, which has large effects on glycogen content in muscle and meat quality. Ten microsatellites and eight genes were mapped. They found that the relative order of genes AE3 and INHA was inverted on the porcine physical map in comparison with the mouse linkage map, but the order of other genes already mapped in the mouse was identical to pigs. Moreover, they found no clear difference between the gene order in pig Chromosome 15 and human Chromosome 2q. Based on the evolutionary link and comparative genomics of animals, it can be determined whether the variation in a gene is or is likely to be associated with a functional trait between closely linked species.

The Examiner also stated in part that "the claims that are broadly drawn to testing any animal for any phenotype associated with polymorphism in the MC4R gene, the art supports the fact that it is highly unpredictable which polymorphisms within the MC4R gene will be associated with which phenotypes." *Id.* at 8-9.

Applicants amended the claims so that they are drawn to testing any animal for a particular phenotype associated with a specific polymorphism in the MC4R gene. Applicants

have shown that a polymorphism in the MC4R gene has been located and this genetic variability is associated with phenotypic differences in meat quality traits. More specifically, Applicants have shown that the marker for improved meat characteristics as evidenced by four meat quality measurements (e.g., pH, marbling, color and drip loss) observed in the specification is allele 2. Because of the evolutionary link between pigs and other meat-producing species, it can be predicted that variation in this gene is also likely to be associated with meat quality in these other species. This polymorphism can be identified in the MC4R gene of these species using the same approach set out in the specification and the resulting single nucleotide polymorphism used for association analysis.

Further, with the advent of techniques to study variation at the DNA level has come the ability to generate large numbers of polymorphic genetic markers in practically any species. Genetic markers have given the ability to track inheritance of linked segments of the genome in suitable pedigrees. A significant association between the inheritance of a particular marker allele and a measured quantitative trait provides evidence that a QTL is linked to the marker in question. By following 100 to 200 markers spread approximately evenly across the genome, in an appropriate pedigree, it is possible to identify all the major QTL influencing variation in a trait in that population. This approach has already been done in plants. This has been followed by a study in livestock which identified genes for controlling differences between wild boar and commercial pigs. Next, followed the identification of QTL within the Holstein cattle population and now by results of many other studies, both within populations and in crosses between populations. Therefore, Applicants respectfully disagree with the Examiner when it comes to saying for enablement of the full scope, requires the use of unpredictable and potentially non-existent products. As stated above, a significant association between the inheritance of a

particular marker allele and a measured quantitative trait provides evidence that a QTL is linked to the marker in question, therefore undefined and undue experimentation is not required to identify which polymorphism, none of which are known other than the disclosed example, have the utility of being associated with favorable meat quality. Moreover, Applicants respectfully submitted it would not be undue experimentation for one of ordinary skill to develop further polymorphisms around the MC4R gene. For a long time, it has been believed that on average, the human genome has one polymorphic position for every 100 positions, and it has been estimated that due to inbreeding in farm animals, that number falls to about 1 in 200 positions on average. The Examiner correctly stated that there is a quite large variation in the average distance from one polymorphism to the next; however, polymorphisms will be useful at least out to a distance of 1 million bp (1mbp) to each side of the MC4R gene and in such a huge region, polymorphisms can be found. This distance is chosen because 1 mbp corresponds to a maximum of 1% crossing over. In each successive generation there is a maximum of 1% chance of separating a marker from the MC4R mutation if these are 1 mbp apart, and using markers on both sides of the MC4R gene simultaneous would lower the risk of false mutation state determination to less than 1 in 10000. Animal breeders are used to error rates much larger than that. Remember that this is the worst-case scenario; polymorphisms should be found much closer than that. Applicants would estimate that others could develop several markers around the MC4R gene in less than three month given the current state of knowledge and a couple of lab technicians – and still without making any independent research as to the effect of any polymorphism on breeding traits.

Recently, an MC4R variant I103 was found to be negatively associated with obesity. These researchers unexpectedly observed a reduced transmission of the I103 allele in 520 obese

trios. They detected 35 heterozygous parents, only 10 of whom transmitted the I103 allele to their offspring (P = .017) suggesting that this allele was protective against obesity. See Geller, et al., Melanocortin-for receptor gene variant I103 is negatively associated with obesity, *Am. J. Hum. Genet.*, 74:572-581, 2004.

While Applicants have amended the claims to animals having the instantly disclosed polymorphism as well as to specific meat quality characteristics associated with the polymorphism; Applicants respectfully request the Examiner take the above statements into consideration.

At page 9 of the Office Action the Examiner stated "the specification has not provided any evidence that polymorphisms linked to this single G ? A change are in fact associated with meat quality traits." "There is no evidence provided that the identified polymorphism is causative of the observed traits. This is a significant absence of evidence, since it is possible that the polymorphism is merely a marker for the causative genotype. In light of the fact that the causative genotype has not been identified, it is unpredictable as to whether or not markers which are linked to the instantly disclosed polymorphism would be informative for the traits of interest herein. " *Id.* at p. 9.

Applicants traversed this rejection. A significant association between the inheritance of a particular marker allele and a measured quantitative trait provides evidence that a QTL is linked to the marker in question. Moreover, Applicants have previously submitted the declaration of Dr. Max Rothschild which addressed the concerns of the Examiner regarding whether the mutation is causative of the genotype. In the declaration, Dr. Plastow demonstrates that the 298N MC4R variant has a functional defect in mediating agonist stimulation as the transfected cells did not accumulate cAMP in response to NDP-aMSH. On page 12 of the declaration and depicted in

Figure 4B, is the effect of the MC4R variant on the muscle fiber type. (See Declaration of April 2, 2002). Dr. Rothschild states "the heterozygous animals were significantly higher in type I fiber % and the type IIa/IIb fiber ratio compared to the 298D homozygous animals. Given low % of type I fiber in the longissimus muscle, the physiological significance of the difference may be doubtful. However, the consistency in increased type I and type IIa fibers from the heterozygous animals can be resulted from the multiple phenotypic effects of the MC4R variant as the MC4R is responsible for the variation in both growth and body fat. Therefore, this MC4R effect on the muscle fiber type might be consistent with the previous results that selection based either leanness or growth in pigs have increased more type IIb fiber types (22)." *Supplemental Amendment* dated April 2, 2002 at p. 12.

Applicants believe they have sufficiently shown that this polymorphism is causative of the observed traits.

The Examiner, however, also stated "even if the declaration did establish it is the lack of accumulation of cAMP in cells, such a showing would not be sufficient to overcome the rejection of record with regard to the scope of the invention including other animals or traits other than those disclosed to be associated with the polymorphism. The declaration is directed solely at the presence of a single mutation in pigs and does not address other animal or other traits." *PTO Paper* dated June 9, 2003 at p. 14.

Applicants amended the claims to animals having the instantly disclosed polymorphism as well as to specific meat quality characteristics associated with the polymorphism. Moreover, it is submitted that the DNA polymorphism shown to be causative of the observed traits, Asp298Asn, in the porcine MC4R gene is in a highly conserved region among other MCR subtypes and other species MC4R protein, therefore it is expected that the different alleles

disclosed will correlate with variability in this gene in other meat producing animals, such as bovine, sheep, chicken, etcetera.

At page 10, the Examiner also stated "applicants have not disclosed how one would go about detecting polymorphisms associated with the traits of interest herein. Because there is no reason to expect that any additional polymorphism is associated with the production of meat with any favorable quality and because of the very large number of possible polymorphisms, screening additional polymorphisms that would be indicators of these traits would require the rearing and subsequent slaughtering of many pigs in order to analyze their meat quality and in order to screen the MC4R gene for informative polymorphisms." "The instantly disclosed polymorphism may be coincident with and unrelated to a different, unlinked (on the chromosome) polymorphism such as another MC4R polymorphism or a polymorphism in an undetermined gene that actually determines meat quality. The instantly disclosed polymorphism would not have any meaning or effect, but might appear to influence litter size due to its close proximity to some other gene." *Id.* at p. 10.

Applicants amended the claims to the instantly disclosed polymorphism in the specification, which is enabling for methods for identifying an animal that possesses a genotype indicative of having favorable meat quality, wherein the DNA is screened for a G to A mutation at position 678 in the MC4R gene. See Spec. at pages 9-11, 13, and 24. Furthermore, once knowledge of the existing polymorphism is known, it takes no more than routine screening to identify the presence of the polymorphism in another species, using the methods disclosed in the application. Thus, this rejection should be withdrawn.

II. CLAIM REJECTIONS - 35 U.S.C. § 102

A. Claims 1, 4-10, 19, 20-24, 29, 32-38 and 40

Claims 1, 4-10, 19, 20-24, 29, 32-38, and 40 were previously rejected under 35 U.S.C. § 102(a) as being anticipated by Rothschild et al. (WO 00/06777). The Examiner stated "the teachings of Rothschild meet the limitations of all the instant claims." *Id.* at p. 12.

Applicants respectfully traversed this rejection. Applicants respectively submitted that WO 00/06777 did not meet all the limitations of the present invention. As stated in Applicants' specification on page 5, this polymorphic site has been described in WO 00/06777. In WO 00/06777, this site was found to be associated with the phenotypic difference in the metabolic traits of fat content, growth rate, and/or feed consumption (see pg. 6, lines 10-12 of WO 00/06777). However, this marker as disclosed in the present invention has now been found to be associate with favorable meat quality characteristics as evidenced by pH, marbling, color and drip loss. Claim 1 was amended to recite the specific traits (pH, marbling, color and drip loss), which are not disclosed, even inherently, in WO 00/06777. Instead, WO 00/06777 is limited to "the metabolic traits of fat content, growth rate, and feed consumption." (See claim 1 of WO 00/06777). Further, the alternate allele in Applicants' present invention, is associated with these beneficial traits. The Examiner stated that "the presence of the aspartic acid codon is allele 1, and this allele is associated with decreased fat content in animals." The marker for the four claimed meat quality measures (level, marbling, color and drip loss) is associated with allele 2. (See specification pg. 9, lines 27-30). The marker for "leanness" as disclosed in WO 00/06777 is identified by the fragments 466, 225, and 76 bp (allele 1) (see pg. 9, lines 14-15 of WO 00/06777). Therefore, Applicants respectively submitted the WO 00/06777 failed to disclose all the limitations of the present invention; thus, the rejection should be withdrawn.

III. CONCLUSION

This Preliminary Amendment accompanies a Continuation Application and is a request under the provision of 37 CFR § 1.136(a) to extend the period for filing a response in the above-identified application for two months from February 9, 2004 to April 9, 2004. Applicant is a large entity; therefore, included in our check is the amount of $420.00 for the two months extension of time. Any deficiency or overpayment should be charged or credited to Deposit Account 26-0084.

Reconsideration and allowance is respectfully requested.

Respectfully submitted,

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